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Citation for published version:

Millar, JK, Mackie, S, Clapcote, SJ, Murdoch, H, Pickard, BS, Christie, S, Muir, WJ, Blackwood, DH, Roder, JC, Houslay, MD & Porteous, DJ 2007, 'Disrupted in schizophrenia 1 and phosphodiesterase 4B: towards an understanding of psychiatric illness' *Journal of Physiology*, vol. 584, no. Pt 2, pp. 401-405. DOI: 10.1113/jphysiol.2007.140210

Digital Object Identifier (DOI):

[10.1113/jphysiol.2007.140210](https://doi.org/10.1113/jphysiol.2007.140210)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Journal of Physiology

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SYMPOSIUM REPORT

Disrupted in schizophrenia 1 and phosphodiesterase 4B: towards an understanding of psychiatric illness

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Disrupted in schizophrenia 1 (DISC1) is one of the most convincing genetic risk factors for major mental illness identified to date. DISC1 interacts directly with phosphodiesterase 4B (PDE4B), an independently identified risk factor for schizophrenia. DISC1–PDE4B complexes are therefore likely to be involved in molecular mechanisms underlying psychiatric illness. PDE4B hydrolyses cAMP and DISC1 may regulate cAMP signalling through modulating PDE4B activity. There is evidence that expression of both genes is altered in some psychiatric patients. Moreover, DISC1 missense mutations that give rise to phenotypes related to schizophrenia and depression in mice are located within binding sites for PDE4B. These mutations reduce the association between DISC1 and PDE4B, and one results in reduced brain PDE4B activity. Altered DISC1–PDE4B interaction may thus underlie the symptoms of some cases of schizophrenia and depression. Factors likely to influence this interaction include expression levels, binding site affinities and the DISC1 and PDE4 isoforms involved. DISC1 and PDE4 isoforms are targeted to specific subcellular locations which may contribute to the compartmentalization of cAMP signalling. Dysregulated cAMP signalling in specific cellular compartments may therefore be a predisposing factor for major mental illness.

(Received 6 July 2007; accepted after revision 31 August 2007; first published online 6 September 2007)

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Schizophrenia is a devastating illness characterized by profound emotional and cognitive disturbances. It is a multifactorial disorder believed to arise, in part, from the actions of multiple genetic variants that influence brain development and function. There is some evidence for clinical and therapeutic overlap between schizophrenia and mood disorders such as bipolar disorder and severe recurrent depression, although this remains a controversial area (for review see Maier *et al.* 2006), and indeed many genetic loci have been implicated in conferring susceptibility to both schizophrenia and affective disorders (for review see Blackwood *et al.* 2007). One such candidate is the Disrupted in schizophrenia (*DISC*) locus which was first implicated in psychiatric illness

by our studies of a large Scottish family in which a balanced t(1;11) chromosomal translocation cosegregates significantly with schizophrenia, bipolar disorder and severe recurrent depression (Blackwood *et al.* 2001). This translocation directly disrupts two overlapping genes named *DISC1* and *DISC2* on chromosome 1 (Millar *et al.* 2000), and statistical analysis indicates that inheritance of the translocation is causal in this family (Blackwood *et al.* 2001). Thus disruption of these genes is likely to generate the risk of severe mental illnesses in translocation carriers.

Since its identification as a genetic risk factor for major mental illness, the *DISC* locus has been implicated in causing schizophrenia, bipolar disorder and unipolar depression in multiple populations worldwide, although these studies highlight different regions and no definitive functional variants have yet been found (for review see Porteous *et al.* 2006). The *DISC* locus is consequently now regarded as a highly convincing candidate for psychiatric illness. Moreover, genetic variants at this locus have been

This report was presented at a symposium on Compartmentalized signalling in neurons, which took place at the Life Sciences 2007 meeting, 9–10 July 2007, Glasgow, UK.

associated with cognitive functions in both affected and unaffected individuals, as well as with influences on hippocampal structure and function, and grey matter volume (for review see Porteous *et al.* 2006). The *DISC* genes are therefore plausible genetic and biological candidates for causing susceptibility to major mental illness and it is now essential to discover the mechanism by which this occurs.

DISC1 encodes multiple isoforms with no significant homology to other proteins. Primary sequence analysis of the full-length ~100 kDa isoform indicates the presence of a head domain with no clear structure, and a long helical tail domain containing multiple regions with high coiled-coil forming potential (Millar *et al.* 2000). *DISC1* therefore has structural similarity to other proteins termed molecular scaffolds, and studies of its very large number of potential binding partners indicate that *DISC1* may indeed act as an assembly point for functional multiprotein complexes. *DISC2*, however, lacks any obvious coding potential and is suggested to belong to the class of antisense RNA genes that act as regulators of sense gene expression (Millar *et al.* 2000), although there is as yet no experimental data to support this speculation.

Most work to date has focused upon *DISC1*, and as part of the drive to identify its function and mechanism of involvement in psychiatric illness, many studies have concentrated upon identification of *DISC1* interacting proteins. Numerous *DISC1* binding partners involved in many cellular functions have been identified, including LIS1 and NDEL1, which implicate *DISC1* in the process of neuronal migration within the developing brain (for review see Mackie *et al.* 2007). Another binding partner is phosphodiesterase 4B (PDE4B) identified in a large-scale yeast two-hybrid screen that identified a substantial number of potential interactors (Camargo *et al.* 2007). PDE4B was selected for detailed follow-up because it had been independently identified as a risk factor through our studies of another Scottish family with a t(1;16) chromosomal translocation (Millar *et al.* 2005). This translocation was inherited by cousins, one diagnosed with schizophrenia and the other with a psychotic disorder, and directly disrupts *PDE4B* on chromosome 1, as well as the gene encoding cadherin 8 (CDH8) on chromosome 16 (Millar *et al.* 2005). PDE4B has subsequently been further implicated as a genetic risk factor (Pickard *et al.* 2007). This interaction between two independently identified risk factors suggests that we have identified a pathway that is likely to directly contribute to the symptoms of severe psychiatric disorders.

PDE4B is an excellent biological candidate in its own right. It belongs to a family of four genes (PDE4A-D) that act to hydrolyse cAMP and switch off cAMP signalling cascades (for review see Houslay & Adams, 2003). PDE4s are orthologous to the *Drosophila melanogaster* *Dunce* gene, which has a critical role in synaptic plasticity,

and learning and memory (for review see Davis, 1996). Moreover inhibitors of PDE4 activity influence NMDA receptor-dependent memory (for review see Mackie *et al.* 2007), while the PDE4 inhibitor rolipram additionally has an antidepressant (for review see Houslay & Adams, 2003) and antipsychotic-like (Kanes *et al.* 2007) profile. Mice deficient in PDE4B and PDE4D have been generated and both lines (untreated) behave similarly to wild-type mice that have been treated with antidepressants (O'Donnell & Zhang, 2004). Finally, several studies have reported that antidepressant treatments up-regulate expression of PDE4 isoforms in rodent brain (for review see Mackie *et al.* 2007). These observations are all consistent with an involvement of PDE4B and other PDE4s in molecular mechanisms that underlie psychiatric disorders.

Through the use of alternative promoters and first exons, the four PDE4 genes encode more than 20 different isoforms, with five separate PDE4B isoforms identified to date (for review see Houslay & Adams, 2003; Cheung *et al.* 2007). PDE4s have a conserved modular structure consisting, in long isoforms, of a C-terminal catalytic domain linked to two regulatory regions termed upstream conserved regions 1 and 2 (UCR1 and UCR2, for review see Houslay & Adams, 2003). In addition each PDE4 isoform has a unique N-terminal domain that is believed to confer targeting to specific subcellular compartments (for review see Houslay & Adams, 2003). Specific isoforms are therefore likely to occupy restricted subcellular locations and this, together with the presence of factors such as A kinase anchoring proteins (AKAPs), is believed to underpin compartmentalized cAMP signalling within cells.

Interaction between *DISC1* and PDE4B has been confirmed, and in addition *DISC1* has been shown to bind isoforms from each PDE4 family (Millar *et al.* 2005; Murdoch *et al.* in press). PDE4 association with *DISC1* involves the common regulatory UCR2 domain, suggesting that *DISC1* may have a role in modulating PDE4 catalytic activity (Millar *et al.* 2005). Moreover, in the human neuroblastoma cell line SH-SY5Y, the 71 kDa isoform of *DISC1* has been shown to associate dynamically with PDE4B, with the complex dissociating in response to elevated cAMP levels in a protein kinase A (PKA)-dependent manner (Millar *et al.* 2005). Under these same conditions PDE4B becomes PKA phosphorylated and its catalytic activity increases (Millar *et al.* 2005). This led to a hypothetical model whereby *DISC1* sequesters PDE4B in a low activity form until it is required to switch off cAMP signalling, whereupon PDE4B is released from *DISC1* in a high activity form.

Subsequent work has revealed that this model is too simplistic, since dissociation in response to elevated cAMP is dependent upon specific *DISC1* and PDE4 isoforms, and some isoform combinations do not dissociate (Murdoch *et al.* 2007). Specifically, full-length 100 kDa *DISC1*

dissociates from PDE4C and PDE4D isoforms, but not from PDE4A or PDE4B isoforms, while the shorter 71 kDa DISC1 isoform does dissociate from PDE4B (Millar *et al.* 2005; Murdoch *et al.* 2007). The basis for the differential responses of specific isoforms to elevated cAMP may relate to the number of contact sites between DISC1 and PDE4. A peptide array strategy revealed that there are in fact five PDE4 binding sites on DISC1 (Murdoch *et al.* 2007). Three of these binding sites are specific for PDE4B isoforms, while two potentially bind isoforms from each PDE4 family (Murdoch *et al.* 2007) and it is therefore possible that 100 kDa DISC1 binds stably to PDE4B because of these additional points of contact, while in the 71 kDa form of DISC1, whose amino acid content has yet to be determined, some of the binding sites may be absent, allowing dissociation to occur. Although the complexities of the DISC1–PDE4 interaction are not yet understood, there is clearly potential for dysregulation of cAMP signalling cascades via altered interaction between these proteins.

The search is now on for evidence that there is indeed dysregulation of DISC1 and PDE4 in psychiatric patients. In lymphoblastoid cell lines derived from family members carrying the t(1;11) translocation that disrupts DISC1, it has been shown that DISC1 expression is reduced to approximately half normal levels suggesting that there is no protein expressed from the disrupted allele (Millar *et al.* 2005). DISC1 haploinsufficiency is therefore likely to underlie the risk of psychiatric illness in individuals carrying this translocation. Consistent with this, DISC1 expression is reportedly reduced in lymphoblastoid cell lines from unrelated bipolar disorder patients (Maeda *et al.* 2006). However, studies of postmortem brain have yet to reveal significantly altered DISC1 expression levels in the limited number of psychiatric patients studied so far (Lipska *et al.* 2006). The t(1;16) translocation that disrupts PDE4B has not yet been fully investigated, but the PDE4B1 isoform is reduced in a lymphoblastoid cell line from these patients (Millar *et al.* 2005). Overall, then, there is evidence that altered expression of both DISC1 and PDE4B is related to the presence of psychiatric symptoms.

Studying dysregulated DISC1–PDE4 interactions in psychiatric patients is complicated by many factors including tissue availability, lifetime drug treatment and other co-morbidities. Mouse models will therefore provide an alternative means to investigate the involvement of DISC1 and PDE4 in molecular mechanisms that cause psychiatric disorders. A natural *DISC1* mutation in all 129 strains of mice was discovered recently (Koike *et al.* 2006; Clapcote & Roder, 2006). A deletion within exon 6 introduces a premature stop codon and is therefore predicted to abolish normal DISC1 protein expression. Unexpectedly however, the majority of DISC1 isoforms are apparently expressed as usual (Ishizuka *et al.* 2007) possibly due to an exon-skipping mechanism. A modified

version of the 129 *DISC1* allele carrying an artificial stop codon in exon 8, and downstream polyadenylation signal, reportedly results in cognitive defects related to schizophrenia when transferred to a C57BL/6 J background. Two ethyl nitrosourea-induced *DISC1* mutations have also been reported (Clapcote *et al.* 2007). These missense mutations are a change of glutamine to leucine at position 31 (Q31L), and a leucine to proline at position 100 (L100P). Detailed analysis revealed that the Q31L and L100P mutations result in phenotypes related to depression and schizophrenia, respectively, using measures designed to correlate in mice with clinical manifestations of these illnesses (Clapcote *et al.* 2007). The intriguing differences in the phenotypes resulting from these two mutations add further weight to the evidence that *DISC1* is a risk factor for both schizophrenia and depression, and indicate that different types of mutations within this gene may cause susceptibility to major mental illness.

Both missense mutations are located within PDE4B-specific binding sites, and both reduce binding between DISC1 and PDE4B in overexpression systems, with the L100P mutation having the greater effect (Clapcote *et al.* 2007). This suggests that altered DISC1–PDE4B binding does indeed confer risk of developing psychiatric illness. Although interaction between DISC1 and PDE4B has yet to be demonstrated in the brain, in Q31L mutants brain PDE4B activity is also reduced, most likely as a direct effect of the mutation upon PDE4B association with DISC1 (Clapcote *et al.* 2007). Reduced PDE4B activity may therefore be a contributory factor in some human cases of depression. This reduced PDE4B catalytic activity of Q31L mutants is apparently at odds with the model proposing that PDE4B exists in a high activity form when not associated with DISC1, implying that the mechanism by which this mutation reduces DISC1–PDE4B association is not related to the sequence of events leading to cAMP-induced PDE4B dissociation from DISC1, and concomitant PDE4B activation. These initial biochemical observations require substantial additional effort to dissect out the pathways by which the missense *DISC1* mutations lead to the observed behavioural and pharmacological phenotypes, with PDE4B being just one of the many DISC1 binding partners awaiting investigation.

In summary, a complex picture is now emerging whereby DISC1 modulates cAMP signalling through its association with PDE4s. Many factors are likely to influence this interaction, including which DISC1 and PDE4 isoforms are involved, their expression levels and their binding site affinities. Studies of psychiatric patients and mouse models suggest that any functional variation affecting these factors may confer risk of developing major mental illness. It is now important to discover which molecular pathways and central nervous system functions are regulated by DISC1–PDE4 interaction and which are dysfunctional in human psychiatric illness. The

former will be determined in part by which additional proteins are associated with, and modulated by, the cAMP signalling cascades governed by DISC1–PDE4 complexes, and such proteins are likely to include some of those identified as potential DISC1 binding partners in yeast two-hybrid screens. An additional critical factor will be compartmentalization of these protein complexes, since specific isoforms of both DISC1 and PDE4 are targeted to particular subcellular compartments including the centrosome, nucleus and mitochondrion (Millar *et al.* 2005; for review see Mackie *et al.* 2007; James *et al.* 2004; Lynch *et al.* 2006). Consequently there is potential for DISC1–PDE4 complexes to modulate aspects of the function of several organelles. There is also evidence that DISC1 and PDE4 isoforms are both present at neuron-specific sites such as synapses (Clapcote *et al.* 2007; Kirkpatrick *et al.* 2006), raising the intriguing possibility that cAMP signalling downstream of synaptic receptors may be abnormal in some psychiatric patients.

DISC1–PDE4 complexes may thus offer new prospects for therapeutic intervention in psychiatric illness. Risperidone has antidepressant and antipsychotic-like action through inhibition of PDE4 activity, indicating that it may be possible to treat psychiatric disorders by directly modulating PDE4-specific cAMP hydrolysis. DISC1 interaction with the PDE4 family of isoforms may therefore offer an exciting new opportunity to provide better treatment for severe and disabling psychiatric illness.

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